

Summary of Discussion Session: EPA Workshop on the SENCAR Mouse in Toxicological Testing

Introduction

At the EPA Workshop on the SENCAR Mouse in Toxicological Testing, held in Cincinnati, Ohio, on May 1 and 2, 1985, a general discussion was held on the afternoon of the second day. All participants were invited to attend. The discussion was chaired by Richard J. Bull, Washington State University, Pullman, Washington. The focus of discussion was the need for further research. Participants were also encouraged to clarify any questions and raise any issues concerning the papers presented during the previous 1½ days.

The discussion fell into six general subject areas: applications of the SENCAR model, regression and progression of papillomas, comparative pathology of SENCAR mice, basis for the increased sensitivity of the SENCAR, use of SENCAR data in regulatory activities, and statistical evaluation of skin tumor data. This paper summarizes the discussion and organizes the remarks into these six areas.

Applications of the SENCAR Model

Dr. R. Bull (Washington State University) commented that there was a problem in using the SENCAR mouse assay across the board as a screening technique because test results indicate that its use is restricted to certain classes of compounds that impact certain target organs. Given that restriction, he said, one should use the SENCAR system as part of a battery of assays for screening. He then asked Dr. S. Nesnow (EPA Carcinogenesis and Metabolism Branch) to discuss the EPA's research into using SENCAR data for risk assessment.

Dr. Nesnow explained that, three years ago, the EPA was actively investigating the potential health hazards of diesel exhaust because of pressure to dieselize the automobile fleet. Since previous studies had shown that these complex mixtures, particularly gasoline and diesel exhausts, were weak carcinogens and would not induce respiratory tumors in a variety of species, EPA researchers had to utilize some alternate method for evaluating the comparative tumorigenic activity of these complex mixtures. The SENCAR mouse skin model was chosen because it had historically been a very good and sensitive system for polycyclics and some other chemical classes. In particular, it seemed to provide good dose-

response data for complex mixtures. Using this system, he continued, EPA researchers found that they could obtain good reproducible dose-response data on tumor incidence that could be quantitatively compared with data from other short-term test systems (e.g., the Ames test, mouse lymphoma gene mutation assay, cell transformation assays) and with the human epidemiology. This comparative potency technique showed a direct relationship between mouse skin tumor initiating activity and human epidemiology (lung cancer) of coke oven emissions, roofing tar emissions, and cigarette smoke. There was an approximately 1,000-fold range of response of these complex mixtures in humans and about an 800-fold range of response in mouse skin. Currently, Dr. Nesnow said, EPA is continuing this line of investigation by testing mixtures of more current interest, such as wood smoke, as well as evaluating additional reference carcinogens for which both mouse skin data and human epidemiology data have been obtained.

In response, Dr. Bull said that he felt there were definite limitations in how broadly this approach could be applied to complex mixtures since there seems to be a fairly high false-negative rate for compounds that would ordinarily impact other target organs. For example, based on his results with aromatic amines, he would be very hesitant to use the SENCAR mouse to judge the hazards of a dye effluent.

Dr. Nesnow agreed with this comment. Specifically he said that researchers must at least know the broad chemical classes of the major components of the mixture, e.g., whether they are polycyclics or whether they are aromatic amines. For example, EPA research has shown that several aromatic amines including α -naphthylamine and 4-aminobiphenyl were totally inactive on mouse skin. Thus, it is essential, he said, that researchers know what class of chemical they are evaluating in order to select the most appropriate test system, be it mouse skin, Ames, mouse lymphoma, or some other system.

Dr. J. Strickland (National Cancer Institute) commented that the SENCAR mouse was derived to be sensitive to skin carcinogenesis by a certain protocol. He said that, based on his knowledge of the literature and the presentations at the workshop, there did not yet appear to be any good biology for increased sensi-

tivity of the SENCAR mouse in any other organ than the skin. He felt that it was important that people interested in using the mouse as a test system recognize this fact.

Regression and Progression of Papillomas

Dr. Bull then moved the discussion onto the subject of regression and progression, particularly the progression to the malignant stage, a subject he felt to be central to the use of the SENCAR mouse in short-term screening. To open this discussion, he posed the following question to Dr. H. Hennings [National Cancer Institute (NCI)]: "Your results would suggest that there are at least two hits involved in complete carcinogenesis and perhaps more. In addition, data you presented at this workshop suggest a possible weakness in the use of a gross papilloma count. These data showed that a longer duration of TPA treatment resulted in a greater proportion of papillomas that then seemed to regress, while a shorter duration of TPA treatment gave a plateau of four or five papillomas per animal but these papillomas seemed to progress more readily to carcinomas. Thus it appears that some papillomas progress preferentially to carcinomas. From the vantage point of the National Cancer Institute, how should this information influence the postures of regulatory agencies with respect to risk assessment models?"

Dr. Hennings agreed that TPA-dependent papillomas are very heterogeneous with respect to progression and that many papillomas do not progress to carcinomas. In light of this fact, he thought that papilloma counts would not be a good basis for making regulatory decisions. Instead he thought it would be better to base such decisions on real carcinogenesis, i.e., the production of carcinomas.

Supporting this point, Dr. Bull reminded the participants of a slide he had presented that showed the ratio of papillomas to carcinomas. The shape of that curve indicated that relatively low papilloma yields had higher conversion rates than higher yields.

Dr. Hennings responded. He reminded participants that there is a limit to how many carcinomas an animal can get before it dies. So, for example, if a mouse has 20 or 30 papillomas, it is not going to get more than 4 or 5 carcinomas. For this reason, mice with a high papilloma incidence will never have a very high conversion rate.

For that reason, Dr. Bull said, his data were limited to groups with 10 or fewer papillomas. However, even in the area of 1 to 3 papillomas, the rate of conversion certainly changed as the papilloma yield increased.

Dr. B. Diwan (NCI Frederick Cancer Research Facility) asked Dr. Hennings what he thought the differences in biochemical reactions (DNA binding, etc.) of normal epithelial cells and initiated cells to NQO or an irritant in culture would be. Dr. Hennings replied that

he would like to develop a malignant conversion assay in cell culture, but that he had not done that yet.

Dr. Nesnow said he thought there were some data in the literature which suggested that both DMBA and BP gave parallel dose-response curves in the tumor initiation protocol when the mice were scored for papillomas at 6 months of treatment or scored for carcinomas after a year of treatment with TPA. This suggested that the papilloma response in the tumor initiation protocol (i.e., the number of papillomas per mouse) could be used to indicate the carcinoma response in the tumor initiation protocol (i.e., the number of mice bearing carcinomas) for certain chemicals.

Dr. Bull indicated that a major reason for exploring this issue was the need to find an economically feasible system for screening potential carcinogens. He thought that use of a papilloma count was almost essential if the SENCAR system was to be economically feasible for screening.

Comparative Pathology of SENCAR Mice

Dr. G. Knutsen (Pathology Associates, Inc.) then posed the question: Does the pathology of the SENCAR mouse vary depending on the source of the mouse (e.g., Harlan, Oak Ridge) and if so, how does it vary?

Dr. A. Klein-Szanto (University of Texas System) replied that Harlan animals did seem to be more sensitive than the original Oak Ridge and the actual Frederick animals. He said that in recent experiments at the University of Texas, Harlan animals had shown a much higher incidence of papillomas and carcinomas and a shorter latency period than the original Oak Ridge animals. Response in the Oak Ridge animals was similar to response in Frederick colony animals. Also, he said, Harlan animals are larger than the Oak Ridge animals.

Dr. W. Baird (Purdue University) said he thought that continual selection was essential with both the Harlan and Oak Ridge stocks to avoid genetic drift that would result in increased variability and decreased sensitivity. For example, he continued, Boutwell (1) recently compared a strain that he had maintained and continually selected with one of the commercial SENCAR strains. He found a small but distinct difference in sensitivity. According to Dr. Baird, this was just one example of the need to conduct DMBA and TPA selection periodically—for example every 1 or 2 years.

Dr. Knutsen said he had seen some differences in the kidney lesions of Harlan and Oak Ridge mice, and asked whether other participants had observed a difference.

Dr. Klein-Szanto commented that his studies had shown a very low incidence of amyloid in the kidneys of TPA-treated, 2-year-old Oak Ridge animals, but that approximately 60 percent of the animals had amyloid in the liver or spleen.

Dr. J. Ward (National Cancer Institute) said he had seen two cases of liver and spleen amyloid and intestinal amyloid in 2 of 30 Harlan mice treated with TPA for 2

years. Aside from that, he said, amyloid in the liver, spleen, and gut was extremely rare in his animals.

Dr. Claudio J. Conti (University of Texas Cancer Center) reported that about 10 percent of approximately 400 untreated SENCAR mice had amyloid in the liver or spleen.

Basis for the Increased Sensitivity of the SENCAR Mouse

Dr. Ward said that the National Cancer Institute had conducted a study with a phthalate using SENCAR and CD-1 mice. First, the phthalate was applied in a continuous promotion two-stage system; then a three-stage system was used with two stages of promotion by TPA followed by the phthalate. This study was positive in the SENCAR and negative after 40 weeks in the CD-1. He asked if anyone at the workshop knew of similar data in which either an initiator or promoter gave consistently positive results in the SENCAR and negative results in other mouse strains. If so, he asked, what does this mean? Could it mean that the SENCAR was abnormally sensitive and therefore the data were not relevant to other mouse strains, much less to the human population?

Dr. Nesnow replied that he had presented several examples of that type of comparative data in his talk and that Dr. T. Slaga (University of Texas) had other data. Dr. Slaga's data indicated that C57 black mice did not respond to TPA as a promoter, while the SENCAR mice did, but that the SENCAR and C57 black mice seemed to be somewhat equivalent in response to some other chemicals including benzo(a)pyrene as a complete carcinogen. Dr. Nesnow said that these data indicate that the SENCAR does seem to be highly sensitive to some but not all polycyclics, particularly DMBA and, to some extent, benzo(a)pyrene. With other chemicals, such as MNNG, however, the SENCAR is not more sensitive.

Dr. Bull reminded participants that the SENCAR was apparently selected to be sensitive to both initiators and promoters. He suggested that, due to this bivariate selection, there might be more than one reason why the SENCAR exhibits increased sensitivity in initiation-promotion experiments. He asked if research should be conducted to look for more subtle differences between strains and whether that was even feasible.

In response, Dr. Baird said he thought it would be better to maintain one inbred strain, as Dr. Slaga had suggested, than to maintain separate stocks selected for sensitivity to initiators or promoters. With several different stocks, he felt that it would be difficult to avoid cross-breeding or in-breeding, and that they would be very costly to maintain.

Dr. Bull said he had heard discussion at the workshop of two possible bases for the sensitivity of the SENCAR: the differentiation initiated by calcium or TPA, and the metabolic differences that Bill Baird identified with PAHs in his experiments (2). He asked if either

or both of those were sufficient to account for the difference in sensitivity, or whether there might be some other factor(s).

Dr. Strickland responded. He said he thought that the SENCAR's increased sensitivity was probably due to more than one factor, one of which was certainly a metabolic component. He cited a recent paper by DiGiovanni (3) which reported that DBA/2 mice were as sensitive as SENCAR mice to initiation with MNNG, a directly acting carcinogen, and promotion with TPA. However, when DMBA was used as the initiator, the SENCAR mice were more sensitive than DBA/2 mice. Thus, in Dr. Strickland's opinion, based on this and other data including his own, it seemed that the SENCAR was better than other strains at activating carcinogens that were not presented in their active form, whereas the SENCAR was no more sensitive to initiation than other mouse strains if the carcinogen was presented in its active form to the basal cells, which are the target cells. However, in order to reach the basal cells, which are at the bottom of the epidermis, the carcinogen must pass through all the upper layers of the epidermis. Thus there may be some practical problems in presenting the carcinogen to the basal cells in *in vivo* experiments that may affect the apparent sensitivity of a strain to a particular carcinogen. In addition, Dr. Strickland continued, initiated SENCAR cells may have a different sensitivity to promoters than initiated cells of other mouse strains, so there may be qualitative differences in the type of initiated cell with respect to promotion.

Dr. G. Carlson (Purdue University) agreed with Dr. Strickland that there was no simple explanation for the increased sensitivity of the SENCAR. He said that the data that he had presented at the workshop and other data that would soon be published showed that, while distribution and binding are very similar in the BALB/c and the SENCAR, the amount of material bound to the skin and to DNA in the tissues varies between the two species, with the BALB/c showing a higher degree of binding. This type of measurement indicates binding to a whole group of cells. Its significance is difficult to interpret, however he said it does not agree with the tumorigenicity data.

Dr. Baird said he thought further study of cell metabolism of the various mouse species in response to initiators and promoters was necessary to elucidate a biological basis for any differences in sensitivity.

Dr. Conti then asked whether anyone had any data concerning the immunology of SENCAR mice. In response, Dr. Strickland said that his laboratory had found the number of epidermal Langerhans cells in fresh epidermal sheets of BALB/c and SENCAR mice to be indistinguishable when examined by three different techniques: ATPase staining, Ia antigen, and Fc receptors. Also, *in vivo* assays at NCI had shown Langerhans cells from adult BALB/c and SENCAR mice to be indistinguishable in terms of allo-antigen presenting ability and contact hypersensitivity. In other studies at NCI, Dr. Strickland continued, SENCAR skin and

BALB/c skin were grafted to nude mouse recipients that were then treated with DMBA initiation and TPA promotion. The SENCAR grafts retained their sensitivity, while the BALB/c grafts retained their resistance, although the sensitivity level of the SENCAR was slightly lower in the graft than it was in the intact animal. In summary, Dr. Strickland said, the immunological and skin graft experiments conducted at NCI indicate no immunological differences between BALB/c and SENCAR.

Dr. Klein-Szanto commented that the immunological parameters reported by Dr. Strickland were all cellular-related. He wondered if anyone had any data on humoral immunological parameters, but there was no response to this remark.

Use of SENCAR Data in Regulatory Activities

Dr. R.W. Niemeier (National Institute for Occupational Safety and Health) said he had been using the C3H/HeJ almost exclusively for assessing complex mixtures. He had just completed a study with CD-1 and C3H mice using asphalt fumes and coal tar pitch that showed asphalt fumes to be highly carcinogenic. He is now investigating the effects of various fractions of asphalt fumes using the C3H/HeJ strain. In addition, he is using the SENCAR strain for some fractions to confirm their increased sensitivity. The response in the SENCAR strain will be compared to the response in the C3H/HeJ strain.

Dr. Ward pointed out that the EPA used approximately 11,000 SENCAR mice in the past 2 years—more than any other institution. He asked what EPA's plans were for future or ongoing studies with the SENCAR, and whether they would continue to use the SENCAR at such a high rate.

Dr. Nesnow agreed that EPA has a major investment in the SENCAR, particularly in the area of air pollution research. He said EPA would definitely continue to evaluate the SENCAR for comparative assessment of complex mixtures and for screening of some pure chemicals, however this continued effort would be smaller than EPA's previous effort, which had involved about 40,000 mice. Dr. Nesnow said he thought the SENCAR mouse was currently very valuable as a standard screening system, however, it seemed, from discussions at the workshop, that more work needed to be done in dosing, pathology, and sensitivity. He said that other government agencies, particularly the National Toxicology Program (NTP), were interested in supporting research with the SENCAR.

Dr. W. Eastin (National Institute of Environmental Health Sciences) confirmed that the National Toxicology Program was interested in using the SENCAR for initiation-promotion studies. He expressed concern about the general lack of published data on the SENCAR strain; however, he said that it was apparent from the workshop that more information is available, but

not yet published. He said the NTP had a very large data base on the B6C3F1 mouse, which it routinely used, and was currently comparing this mouse with the Swiss CD-1 and the SENCAR in an initiation/promotion study. Dr. Eastin asked the participants what they considered to be the most appropriate kinds of controls in initiation/promotion studies using the SENCAR, given that there may be a major difference in stocks from the two major suppliers.

In response to this question, Dr. R. M. Kovatch (Pathology Associates, Inc.) suggested that the NTP use the same approach they had originally used for the B6C3F1 mouse, i.e., room controls which were eliminated from the experiments when they were no longer felt to be necessary.

Dr. Bull said the EPA had always run nonpromoted vehicle controls in its experiments. These controls had received acetone and had been shaved. Although these controls were not, strictly speaking, untreated, there was much data on them that could indicate what background is.

Dr. Klein-Szanto said that, based on the proceedings of the workshop, the pathology of the two original stocks (Oak Ridge and Frederick) was apparently well characterized, whereas there was much less data on the Harlan stock. He said he had data on approximately 500 untreated Oak Ridge mice.

Dr. Knutsen said his work was exclusively with Harlan mice but he did not feel his data could provide any type of baseline at this point.

Dr. Ward said he currently had data on 12-month-old mice and would have data on 2-year-old mice in a few weeks. However he recommended that each institute individually characterize their controls under the unique environmental conditions (feed, caging, hair clipping, etc.) of their facility. That data could then be used for comparison purposes.

Statistical Evaluation of the Skin Tumor Data

Dr. Baird then moved onto the subject of statistical analysis. He asked what was needed to get a good statistical analysis of the data in the SENCAR assay.

Dr. J. Stober (EPA Health Effects Research Laboratory) responded. As the first phase in the statistical design evaluation, she recommended looking at the control data to determine the spontaneous rate. Second, she recommended standardizing the criteria for judging whether or not there had been a response. It is essential, she said, that researchers present these criteria—for example, how they determined whether a lesion is a papilloma or a carcinoma, and whether scoring is being done objectively by the gross lesion or microscopically—so that the SENCAR assay can be objectively evaluated and, more importantly, so that results can be compared across time or between researchers. Finally, she said the number of animals used directly affects the value of the results. In particular, studies where many

animals would be lost due to the toxicity of the compound would require a large number of animals to determine marginal response.

Concluding Remarks

Dr. Bull closed the discussion by thanking Merrel Robinson for organizing the symposium. He reminded participants not to forget the advantages of the SENCAR despite the number of critical comments made at the symposium: that it is a healthy, hardy mouse that compares favorably in lifespan to other stocks of mice,

is sensitive to carcinogens, and relatively easy to work with.

REFERENCES

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